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Current Opinion in Microbiology

Elucidation of host–pathogen protein–protein interactions to uncover mechanisms of host cell rewiring

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Infectious diseases are the result of molecular cross-talks between hosts and their pathogens. These cross-talks are in part mediated by host–pathogen protein–protein interactions (HP-PPI). HP-PPI play crucial roles in infections, as they may tilt the balance either in favor of the pathogens' spread or their clearance. The identification of host proteins targeted by viral or bacterial pathogenic proteins necessary for the infection can provide insights into their underlying molecular mechanisms of pathogenicity, and potentially even single out pharmacological intervention targets. Here, we review the available methods to study HP-PPI, with a focus on recent mass spectrometry based methods to decipher bacterial–human infectious diseases and examine their relevance in uncovering host cell rewiring by pathogens.

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Introduction to host–pathogen protein–protein interactions

Infectious diseases reflect the evolutionary balance between a host and its pathogen. In order to ensure their survival and propagation, pathogens have developed numerous intricate tools to subvert their hosts' defense mechanisms. Understanding how pathogens actively rewire host cell defenses is of particular interest in infectious disease research. Ultimately by identifying host-directed targets for pharmacological intervention, this field of research may contribute to eradicate the public health burden caused by these agents.

The molecular mechanisms underlying pathogenic rewiring of host cells are widely varied. However, as protein complexes and their interaction networks into which they are organized comprise the primary functional modules of the cell [1], we can predict that the disruption of these host networks are likely to be a key strategy for manipulation by pathogens. Re-wiring of the host's proteome, also known as pathogenic hijacking, generally includes intervention at multiple stages of signaling pathways and cellular functions to ensure the robustness of the virulent intervention [2]. This hijacking by protein–protein interactions may be carried out by evolutionarily derived partial molecular mimicry [3], which consists of virulent proteins having evolved similar structures or motifs to the host proteins to mediate such HP-PPI. It has further been proposed that the phenotypic impact of a pathogen is directly proportional to its ability to rewire the host interactome, and that the impacts of individual virulent proteins are linked to their number of interactions with host proteins [4••]. Thus, mapping the host–pathogen protein interactome may provide valuable insights into the biological functions of virulence factor proteins, highlight interactions critical to the pathogens' progression and spread, and improve our overall understanding on the molecular basis of pathogenicity.

In this review, we aim to summarize the methods available to characterize HP-PPI, consider their utility by providing biological insights, and present some outlook into the how the field may develop going forward. Even though we are primarily concerned with the possibilities of characterizing HP-PPI from the perspective of bacterial pathogens, a survey of the literature indicates that significantly more work has been done for viruses in this area [5]. As such, an examination of lessons learned from studies of interactions between viruses and hosts should also be instructive.

It is well established that due to their minimal genomes and by being obligate parasites, viruses rely on HP-PPI as a mean to carry out the pleiotropic functions of their proteins by hijacking various host protein modules to either avoid their clearance or enable their spread. For example, by mapping the Influenza A–human PPI network, viral proteins were reported to be highly interconnected thus forming functional modules, and to interact with a greater number of host proteins compared

to the average degree of connectivity in the human interactome [6]. The HP-PPI map further enabled the identification of multiple molecular mechanisms employed by the virus to manipulate its host, including how Influenza proteins intervene in the WNT/β-catenin pathways as a mean to modulate the host's interferon production [6].

Unfortunately, the characterization of bacterial HP-PPI has lagged behind. The reason for this disparity most likely reflects differences in feasibility. That is, testing all proteins produced by a viral genome for interactions with a host proteome requires significantly less effort than that for bacterial genomes due to their increased genomic complexity. Nonetheless there is increasing amount of evidence that bacteria also rewire host cellular pathways via HP-PPI [2]. Pathogenic bacteria can interact with their host's proteome by three main mechanisms. First, bacterial membrane proteins are an obvious interaction point, as they are located at the physical interface between both organisms. Secondly, bacteria might secrete effector proteins (also known as virulence factors) into the host cell where they can interact with the host proteome. Secreted effector proteins are of particular interest as they are frequently required for full virulence [7]. Additionally, some bacterial pathogens such as certain *Shigella dysenteriae* or *Escherichia coli* strains express Shiga toxins generally during their lytic cycle [8] or release these toxins through Outer Membrane Vesicles during their growth phase [9], leading to the inhibition of protein synthesis or activation of the apoptotic pathways of their host cells. As the number of bacterial host–pathogen interaction studies increases, they demonstrate that while bacteria generally do not rely on host cell machinery for the purpose of replication as directly as viruses do, they do seem to disrupt the immune response [10] and interact preferentially with the hosts' cytoskeleton as a mean of motility, invasion of the host tissues [11] and escape of phagocytic cells [12]. For instance, *Mycobacterium tuberculosis* (Mtb), an intracellular parasite, is known to modulate the host's immune response and prevent its bacterial clearance by suppression of autophagy. Recent work has shown that a secreted Mtb factor, PE_PGRS47, locates in the host's cytosol and inhibits the Major Histocompatibility Complex II mediated antigen presentation, thereby partially suppressing the autophagy of the Mtb containing macrophages in chronic stages of infections [13]. By mapping such host interactors, HP-PPI studies could hint us towards the molecular mechanisms behind certain virulence factors like this PE-PGRS47. In this review, we will describe the available methodologies to achieve such goals and discuss their impact on mechanistic understanding or host cell rewiring.

Protein–Protein Interactions detection methods

Yeast2Hybrid

Historically first, the Yeast Two Hybrid (Y2H) method has been extensively used to detect direct physical interactions between two ectopically expressed tagged proteins in yeasts [14]. Although this method generates direct binary interaction datasets at high throughput, the need for exhaustive screens hampers its feasibility, and its technical challenges such as the non-physiological expression system provokes high rates of false negatives [15]. Nonetheless, many studies in the field of infectious diseases have successfully employed Y2H screens to investigate (near) genome-wide virus–host interactions [16–23], to compare homologous viral proteins from various strains [24,25], or to systematically map bacterial effector proteins–host interactions [26,27•,28–31] (see Supplementary Table 1). In the context of *M. tuberculosis* infections, a Y2H screen along with functional validations, enabled the discovery of a molecular mechanism by which an effector protein, named EsxH, targets the Endosomal Sorting Complexes Required for Transport (ESCRT) necessary for endosomal membrane trafficking, thereby impairing the phagosomal maturation and fusion with the lysosomes [28].

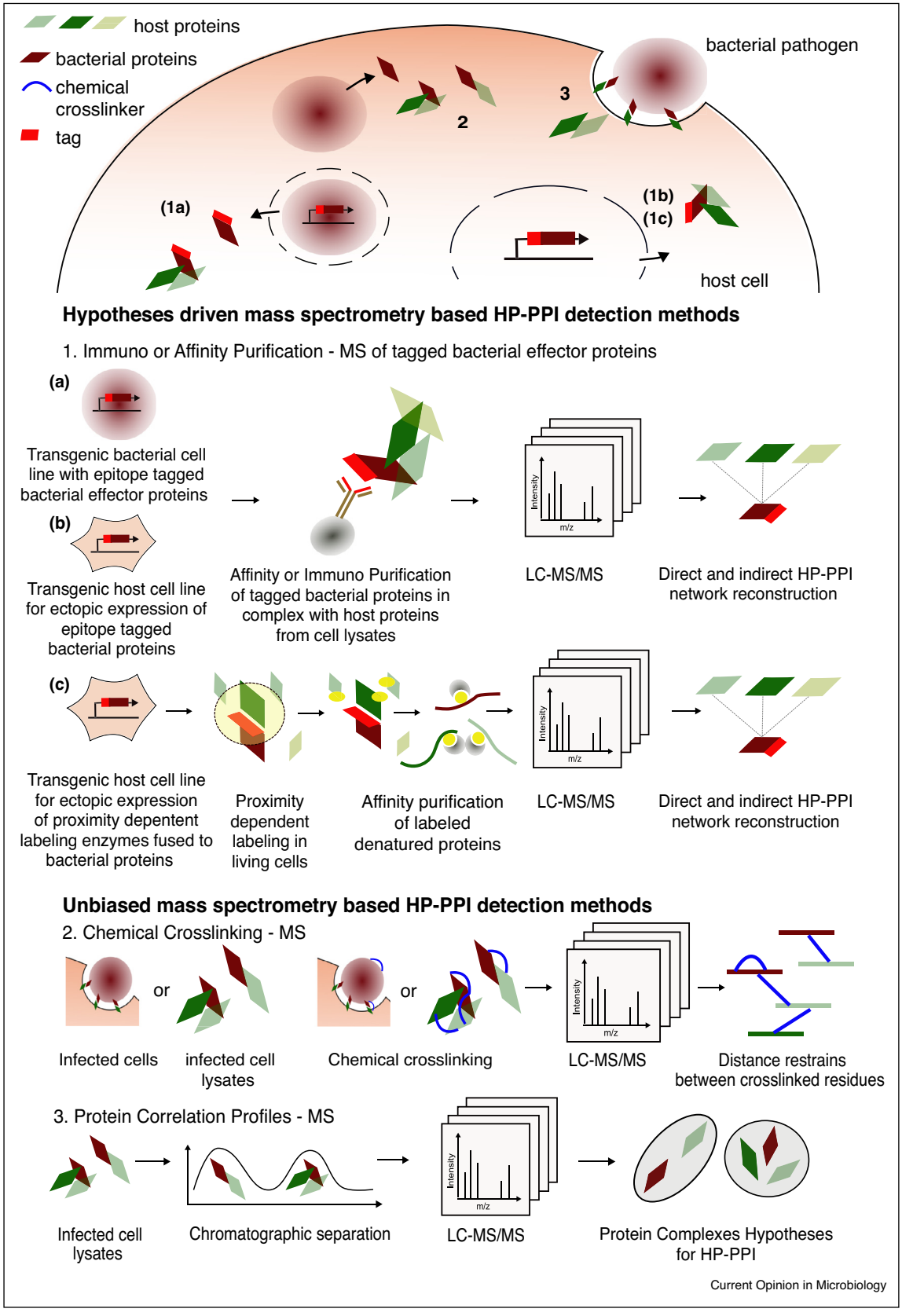
Affinity purification-mass spectrometry and immuno purification-mass spectrometry

In the past two decades, improvements of mass spectrometry (MS) based proteomics in combination with affinity purification (AP) methods have enabled the systematic detection of PPI in near physiological environments [32] (see Figure 1). Most commonly, it consists of fusing an affinity epitope tag to a bait protein, followed by a single or double biochemical affinity-purification or immunopurification (IP) steps in native lysis conditions. The purified bait, along with the non-covalently bound interacting proteins or macromolecular protein complexes (preys), are then identified and quantified via standard bottom up proteomics. To filter out non-specific interactions, this strategy relies on quantitative comparisons with control purifications.

In the field of infectious diseases, AP-MS is commonly applied to systematically map the interactome of individual virulent proteins ectopically expressed in the host's environment [24,33•,34••,35••,36–44], to monitor single virulent proteins [45] or upon infection [46] (see Supplementary Table 1). A related strategy uses immobilized recombinant bacterial effectors on beads [47•] combined with AP-MS from their incubation with human plasma.

Although expressing single virulent genes in host environments is informative, it is believed that during the course of infections, the host–pathogen interactomes undergo infection stage-dependent dynamic changes [48], influenced by the hosts' responses and by the other co-expressed virulent proteins. Thus, some groups have generated replication-competent, epitope tagged viruses which enabled the spatio-temporal monitoring of empirical and quantitative changes upon viral infectious of host

Figure 1


host proteins
bacterial proteins
chemical crosslinker
tag
bacterial pathogen
host cell
(1a)
(1b)
(1c)
2
3
Hypotheses driven mass spectrometry based HP-PPI detection methods
1. Immuno or Affinity Purification - MS of tagged bacterial effector proteins
(a)
Transgenic bacterial cell line with epitope tagged bacterial effector proteins
(b)
Transgenic host cell line for ectopic expression of epitope tagged bacterial proteins
Affinity or Immuno Purification of tagged bacterial proteins in complex with host proteins from cell lysates
Intensity
m/z
LC-MS/MS
Direct and indirect HP-PPI network reconstruction
(c)
Transgenic host cell line for ectopic expression of proximity depentent labeling enzymes fused to bacterial proteins
Proximity dependent labeling in living cells
Affinity purification of labeled denatured proteins
LC-MS/MS
Direct and indirect HP-PPI network reconstruction
Unbiased mass spectrometry based HP-PPI detection methods
2. Chemical Crosslinking - MS
or
or
Infected cells
infected cell lysates
Chemical crosslinking
LC-MS/MS
Distance restrains between crosslinked residues
3. Protein Correlation Profiles - MS
Infected cell lysates
Chromatographic separation
LC-MS/MS
Protein Complexes Hypotheses for HP-PPI
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cells, including for Alphavirus Sindbis [48] and most recently HIV infected human cells [49••]. On the basis of similar principles, Mousnier *et al.* and subsequently So EC *et al.* respectively developed and applied a double purification based method coupled to mass spectrometry to enable the identification of HP-PPI of bacterial effector proteins in host cells upon infections of *Legionella pneumophila*. This study, amongst others findings, described how three effector proteins may target up to 25 Rab GTPases individually during the course of infections [50,51] (see Supplementary Table 1).

Proximity dependent labeling-mass spectrometry

BioID has recently emerged as a new possibility to detect transient and weaker PPI [52] complementary to AP-MS [53]. This method relies on the fusion of a mutated promiscuous biotin ligase BirA* to the bait protein. During an incubation with high biotin concentrations, neighboring proteins to the fused BirA*-bait protein undergo proximity dependent biotinylation reactions. Biotin-conjugated proteins, potential direct or indirect interactors of the bait, can then be affinity purified using streptavidin-coated affinity matrices and quantified by mass spectrometry (see Figure 1). Because the identification of interactions does not depend on the native purification conditions, weak, transient and insoluble interactions such as for membrane proteins can be readily identified [52]. BioID has been applied as a mean to obtain comprehensive interactome information of selected bacterial proteins [54•] belonging to the human pathogen, *Chlamydia psittaci* (see Supplementary Table 1). A variation of this proximity labeling strategy, called APEX, enables much faster reaction times (~30 s), and opens up the possibility of time-resolved proximity measurements [55].

Chemical crosslinking-mass spectrometry

Chemical crosslinking coupled to mass spectrometry (XL-MS) consists of chemically crosslinking proximal reactive side chains of exposed specific amino acids from native proteins in monomeric states or in protein complexes, followed by an MS based, bottom up approach to identify the crosslinked peptides and infer their proteins. XL-MS thus yields fixed distance restraints between bound residues, suggesting direct physical intra-protein or inter-protein interactions between crosslinked peptides belonging to the same or distinct proteins respectively [56] (see Figure 1). Chemical crosslinking reactions can be performed on purified protein samples [57] using GFP epitope tags [58], on cell lysates [59] or on living cells such as on the pathogen *Pseudomonas aeruginosa* [60]. Although having gained popularity in recent years to study the topology of protein networks, decipher the architecture of macromolecular complexes, and provide insights into domain-resolution protein interactions, XL-MS has not yet been widely applied to study HP interactions due to its challenging utilization. One exception is the unbiased study of live human epithelial H292 cells infected with *A. baumannii* which led to the identification of 46 HP-PPI [61••] (see Supplementary Table 1).

Protein microarray based technologies

Membrane proteins play pivotal roles in infections by mediating host–pathogen recognition, docking, adhesion, invasion and secretion. Regrettably, their lack of solubility and their necessity of remaining in lipid-rich environment highly impair their interactome mapping via conventional methods such as AP-MS. To overcome these challenges, Glick *et al.* introduced a screening method for HP interactions, adequate for transmembrane proteins [62••] named the human Membrane Protein Array (MPA). Similarly, several studies have developed and applied Protein Micro Array technologies [63•] including Nucleic Acid Programmable [64] or AVEXIS (AVidity-based Extracellular Interaction Screen) [65,66] to study soluble and transmembrane HP interactions (see Supplementary Table 1).

Discussion

There are a variety of methods available for detecting PPI, where each of them may be applied to answer different questions and come with their own advantages or disadvantages (see Table 1). Many methods have been successfully applied to the HP-PPI field and lead to the discovery of important biological insights. For instance, although human host interactors of viruses and bacteria range across all biological functions, common or pathogen specific themes can be observed within pathogenic groups by meta-analysis of HP-PPI studies. First, viral proteins and to a lesser extent secreted bacterial effector proteins [30], are both more likely to interact with host hub proteins (highly connected proteins in the host network) [16,17,21] and bottleneck proteins (central proteins to many signaling pathways) [10,31,67] for an increased efficiency in altering host cellular processes. Secondly, by performing gene ontology enrichment analysis on the host targets, viral pathogens seems to unavoidably disturb cellular processes as they rely on

(Figure 1 Legend) Mass spectrometry based methods for host–pathogen protein–protein interactions detections in the context of bacterial infections. AP/IP-MS from epitope tagged bacterial effector proteins **(a)** post infections of their host cells enables the identification of physiological HP-PPI. Along with other hypotheses driven methods such as ectopic expressions of tagged bacterial proteins in the host environment **(b,c)**, they can lead to near comprehensive identifications of HP-PPI. However, because they rely on the prior tagging of the proteins of interest, they are limited by the number of proteins that can be cloned and expressed in the relevant cellular systems. Chemical Crosslinking — MS (2) and Protein Correlation Profiles — MS (3) methods, although less sensitive, do not require prior knowledge and tagging of bacterial proteins and thus allow *de novo* discovery of physiological and endogenous HP-PPI directly from infected cells (2 and 3).

Table 1

An overview of the main advantages and disadvantages for the commonly used HP-PPI detection methods. Although Y2H and protein microarray based technologies are high throughput and could theoretically test any gene combinations, they are based on non-physiological experimental conditions and may identify only binary PPI. Techniques such as AP-MS and proximity-dependent labeling coupled to MS, on the other hand, generate physiologically relevant PPI with information about the Post Translational Information (PTM) states of the identified prey proteins and can detect entire protein complexes. Similarly, but in an unbiased manner as it may detect proteome-wide PPI without the need of prior genetic engineering of the pathogens or host cells, PCP-MS and XL-MS on infected cells may detect *de novo* HP-PPI in physiologically relevant conditions. Furthermore, mass spectrometry based methods may be coupled to quantitative proteomics to monitor in a time course-compatible manner, qualitative and quantitative changes of proteins complexes between biological conditions. Unfortunately, the sensitivity of proteome-wide HP-PPI like PCP-MS and XL-MS remain their largest drawback and would probably never gain the same sensitivity as for more targeted methods like AP-MS to study specific protein complexes. Lastly, although XL-MS may be applied on purified protein complexes and provide valuable information their structural arrangements and topologies, it requires high amounts of purified protein complexes and the data analysis remains challenging

	Yeast 2 Hybrid (Y2H)	
Advantages	High throughput • existing human and pathogen ORFeome collections • universality — any cDNA from any protein is testable	
Dis-advantages	Need for exhaustive screens • non-physiological experimental conditions • detects only binary interactions • no PTM information	
	Affinity purification-mass spectrometry (AP-MS)	
Advantages	High throughput • sensitive • detects entire protein complexes • PTM sensitive • when using antibodies against the bait of interest, can be applied from infected tissues directly	
Dis-advantages	Need for transgenic cell lines • needs additional experimental data to distinguish direct from indirect interactors • the identification of PPI depends on the biochemical extraction conditions	
	Chemical crosslinking-mass spectrometry (XL-MS) on:	
	Purified protein complexes	Infected cells or infected cell lysates
Advantages	Provides information on interacting protein domains • residue to residue resolution Information on the topology and structural arrangement of protein complexes	Whole-proteome • adequate for soluble and membrane proteins • can be applied on infected tissues directly
Dis-advantages	For predefined and purified protein complexes only • need for large amounts of purified protein complexes • complex data acquisition and analysis	Low sensitivity/resolution • complex data acquisition and analysis
	Proximity dependent labeling strategies-mass spectrometry	
	BioID	Ascorbate Peroxidase-based Proximity Tagging (APEX)
Advantages	Sensitive • appropriate for weak and transient interactions • adequate for resolving the spatial organizations of the tagged proteins • identification of PPI does not depend on the biochemical extraction conditions Suitable for soluble and transmembrane proteins	Fast reaction times, amenable for time course experiments for temporal resolutions
Dis-advantages	Long reaction times, not suitable for time course experiments Hard to distinguish direct from indirect/proximal interactors	So far applicable to membrane proteins only
	Protein microarray based technologies	
	Nucleic Acid Programmable Protein Array (NAPPA)	Human Membrane Protein Array (hMPA)
Advantages	High throughput • universality — any cDNA from any protein is testable • no need for protein purification compared to classical protein microarrays • gene size does not seem to affect its final intensity	High throughput • physiological for membrane proteins • recognition against the entire pathogen • naturally occurring PTM on the surface of tested pathogen
Dis-advantages	Non-physiological • only binary interactions are detected • no PTM information	For membrane proteins only • no PTM on the expressed protein
	Protein Correlation Profiling (PCP)	
Advantages	Whole proteome studies • unbiased • stoichiometric and quantitative information readily available	
Dis-advantages	Dynamic range of protein abundances between host and pathogen might be too important • low sensitivity • hard to detect kiss and run interactions	

the transcriptional machinery, whereas bacteria tend to mesh with the immune response to prevent their clearance [10]. Thirdly, the manipulation of the host ubiquitin pathways by viruses [68] and bacterial effector proteins [69] is a recurrent finding. By controlling protein degradation and cell signaling, ubiquitination is a critical regulator of various cellular processes such as inflammatory responses, vesicular trafficking and cell cycle, altogether making it an ideal target to hijack for bacterial and viral pathogenicity. Indeed, there is increasing amounts of evidence that numerous human bacterial pathogens hijack and modulate the host ubiquitin processes utilizing molecular mimicry to impair the hosts' defense systems, including the ubiquitin-dependent autophagy, the NF-κB and the inflammatory signaling pathways [70,71].

Numerous approaches could be employed to further improve the quality and completeness of HP-PPI networks. These include combining orthogonal PPI detection methods [53,72,73], considering strain specific variation in dependence on the host cellular modules [74], to acknowledge the genetic diversity of both hosts and pathogens [75] and to beware of host cell-type dependent HP-PPI [33•]. The use of more physiological systems for studying these interactions is also a proximal goal, such as adopting more disease relevant cells or transgenic animal models for the ectopic expression of tagged pathogenic proteins. Likewise, employing infection systems where the virulent proteins are tagged within the pathogen could provide dynamic and more physiological maps of the HP-PPI.

The systematic study of bacterial–host interactions brings additional challenges. The first is to identify all secreted proteins upon infection, where *in silico* predictions and experimental findings do not always corroborate [76]. To help the identification of virulence factors from membrane-contained intracellular bacterial pathogens, one could consider purifying intact pathogen-containing compartments or vacuoles, and characterizing their proteome by mass-spectrometry to find new virulence factors that associate with the host membranes [77]. Secondly, due to their increased genomic complexity compared to viruses, the generation of transgenic cell lines to ectopically express each putative secreted protein would be highly time-consuming. Thirdly, bacterial systems generally lack adequate genetic tools preventing endogenous tagging of their secreted proteins. Thusly, we hypothesize that more global approaches for bacterial–host PPI detection may be useful. In the last years, numerous groups have been working towards developing methods which do not require genetically engineered cells to systematically identify in an unbiased manner endogenous protein complexes in physiological samples by correlating protein profiles (PCP-MS) across various biochemical separations or chromatographic techniques [78,79]. Not only does this mass-spectrometry based approach yields lists of putative protein complexes, but it also reports stoichiometric and quantitative information for the identified components. Unfortunately, despite tremendous improvements in the field, the sensitivity remains the limiting factor. It is especially problematic in infectious diseases, where the dynamic ranges in terms of protein abundances from pathogenic organisms are generally several orders of magnitude lower than those of the host proteome [80].

In any case, regardless of which methods were employed, it is imperative to validate and functionally characterize the discovered HP-PPI to understand how they impact the course of infections. To do so, interaction studies can be coupled to endogenous host interaction maps [81,82] or to functional genomic screens to measure the fitness cost upon disruptions of either pathogenic or host molecular components [21], as was done for the HCV interactome [33•]. By measuring sets of phenotypes such as pathogenic replication or host cell death, functional studies have the benefice of being able to simultaneously identify the positive, negative or neutral impact on the infection of targeted bacterial [83] or host factors [74,84]. Altogether, interaction studies, biochemical characterizations and functional screens may not only identify host–pathogen interactions, but also inform us on their phenotypic impacts and their molecular mechanisms for bacterial or viral pathogenicity.

Conflicts of interest

None.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.mib.2017.07.005.

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